Visualization of platinum accumulation by synchrotron radiation X-ray fluorescence in cancer tumor of patients treated with oxaliplatin-based chemotherapy

Oxaliplatin (trans-(±)-diaminocyclohexane oxalatoplatinum; L-OHP), a platinum-based drug, is a key chemotherapeutic agent for colorectal cancer (CRC). Oxaliplatin is a third-generation platinum agent that forms cross-linking adducts, thus blocking DNA replication and transcription [1], similarly to cisplatin and carboplatin. However, there are major problems regarding oxaliplatin-related toxic effects, particularly, peripheral sensory neuropathy being the important dose-limiting toxicity of oxaliplatin-based therapy. Therefore, a prediction of the efficacy and toxicity of oxaliplatin-based chemotherapy may possibly improve its efficacy and safety in patients with CRC.

Synchrotron radiation X-ray fluorescence spectrometry (SR-XRF) traces both the chemical elements originally present in human tissues, such as potassium, calcium, zinc, copper, and iron, and the elements contained in drugs, such as noble metals. However, because of the extremely low concentration of such elements in human tissues, the *in situ* visualization of the distribution of the elements has not been possible using conventional imaging techniques. We applied SR-XRF to visualize the distribution of platinum and other elements in rectal cancer specimens resected from patients who received oxaliplatin-based preoperative chemotherapy. We also evaluated the correlations of Pt levels with therapeutic effects and clinicopathologic factors.

Our study subjects consisted of 30 patients who underwent surgical resection of rectal cancer at the Department of Surgery and Oncology, Kyushu University Hospital (Fukuoka, Japan) between January 2005 and December 2014. All patients received oxaliplatin-based chemotherapy without radiotherapy. Primary tumor response was assessed by physicians on the basis of their comprehensive interpretation of CT, MRI, and colonoscopy findings and reported in accordance with the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines [2]. Neurologic toxicity was assessed in accordance with the Neurotoxicity Criteria of Debiopharm (DEB-NTC) [3] and toxic effects other than neurotoxicity were evaluated referring to the National Cancer Institute-Common Toxicity Criteria (NCI-CTC), version 4.0.

All resected specimens were fixed in formalin and embedded in paraffin, and all tissues adjacent to the specimens were evaluated histologically using the World Health Organization criteria. Consecutive 5-μm formalin-fixed, paraffin-embedded sections were cut and placed onto glass slides. The paraffin-embedded slices were stained with hematoxylin and eosin (H&E) and exfoliated from the slide glass using a cell transfer technique with Malinol mounting medium (Muto Pure Chemical, Tokyo, Japan) [4]. Then, this peeled slice was used for XRF analysis. The remaining paraffin-embedded slices were subjected to immunohistochemical analyses to identify the tumor region (stained for E-cadherin), the stromal region (stained for α-SMA), and tissue degeneration after chemotherapy (stained for CC-3).

SR-XRF measurements of rectal cancer specimens were performed at SPring-8 **BL37XU**. The monochromatic X-ray beam at 14.5 keV was focused into a 0.5 μm diameter using a Kirkpatrick-Baez type X-ray focusing system. The spatial resolution in our measurement was 0.5 μm, and 1.5 h was required to obtain the 2-mm-square tumor image for a sample mounted on an XY-scanning stage (excitation time of 0.1 s, 10 μm steps) [5].

Standard solutions were used to create the calibration curves for Pt, Cu, Zn, and Fe. The elemental concentration was calculated from the integrated elemental intensity using the calibration curve. The detection limit (DL) of each element was defined as the concentration at which the detected peak intensity could be statistically distinguished from the random fluctuations of the corresponding background at a confidence level of 3 standard deviations (SD). As a result, the DLs of Pt, Cu, Zn, and Fe were estimated to be 1.848, 0.380, 1.331, and 3.403 ppm, respectively. In our study, five areas in sections of SR-XRF images, each 50 μm square, were randomly selected, and the average concentrations of the elements were calculated.

As shown in Fig. 1, the Pt concentration in rectal cancer tissue could be estimated to be in the range of 2.85 to 11.44 ppm. In the tumor epithelium, the Pt concentration was significantly higher in areas of degeneration caused by chemotherapy than in the nondegenerated area ($p < 0.001$). Conversely, in the tumor stroma, the Pt concentration was significantly higher in patients with limited therapeutic responses than in those with strong therapeutic responses ($p < 0.001$). In our study, the Pt accumulation in the tumor stroma significantly correlated with histological chemoresistance to oxaliplatin-based chemotherapy. Therefore, our study suggests that drug accumulation in the tumor stroma possibly inhibits oxaliplatin delivery to the tumor cells.

In conclusion, we used SR-XRF to visualize and quantify, for the first time, the distribution of Pt and trace elements in resected rectal cancer tumor specimens from patients treated with oxaliplatin-based chemotherapy. Our novel results indicated that the Pt concentration in tumor stroma is significantly associated with therapeutic response in rectal cancer, suggesting that drug accumulation in the tumor stroma is one possible cause of platinum resistance. The examination of Pt accumulation in the biopsy specimen of rectal cancer during Pt-containing chemotherapy might be useful to predict the histological response and to avoid further administration of this agent to patients with poor-response tumors.

This study was approved by the Kyushu University Hospital Human Research Ethics Committee and conducted in accordance with the Ethical Guidelines for Human Genome/Gene Research enacted by the Japanese Government and the Declaration of Helsinki. Informed consent to harvest tissue for the studies was obtained from all patients.

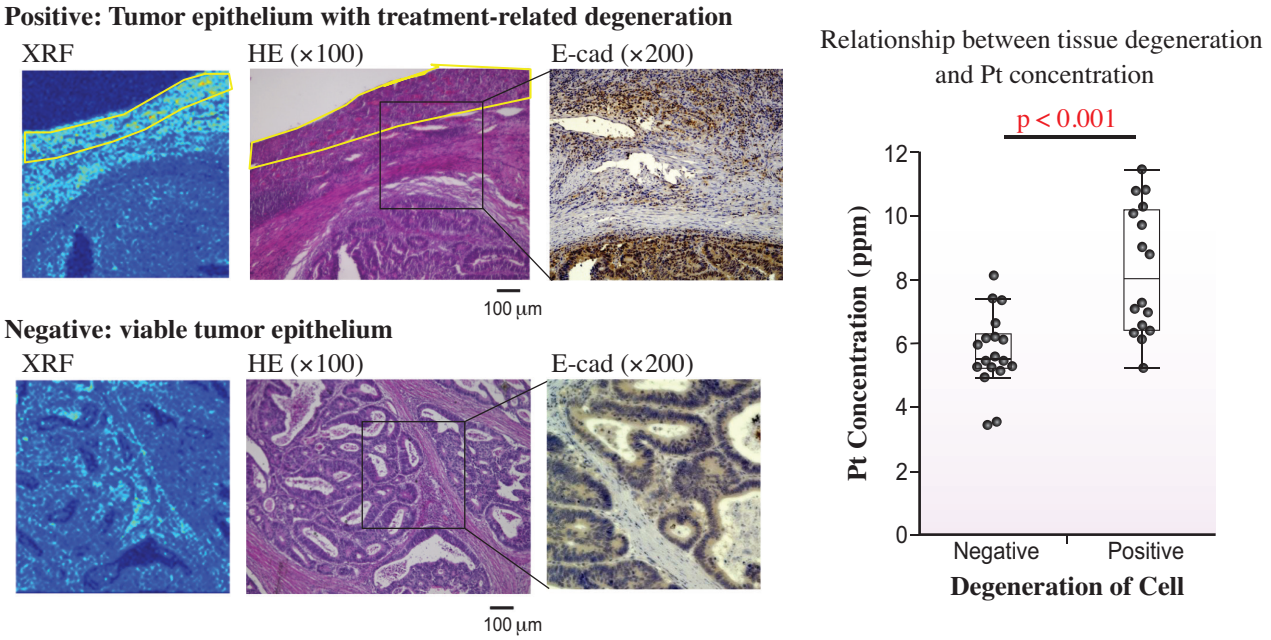

Fig. 1. Tumor epithelial tissue degeneration and platinum concentration. There is a high concentration of platinum in the therapeutically effective part of the tumor epithelium.

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